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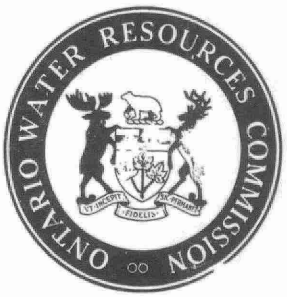

RESEARCH
PUBLICATION
NO. 9

VIRUS REDUCTION

IN THE

WASTE STABILIZATION POND

THE ONTARIO WATER RESOURCES COMMISSION

VIRUS REDUCTION

IN THE

WASTE STABILIZATION POND

By:

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June, 1966

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Publication No. 9

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VIRUS REDUCTION IN THE OXIDATION LAGOON

INTRODUCTION

The increasing acceptance of the "oxidation lagoon" or "stabilization pond" as a method of domestic sewage treatment has been criticized, in part, relative to the efficacy of the system to reduce pathenogenic virus levels in sewage. McAnulty (1) considers the "pond" to be only a primary treatment process, and as such, according to Kabler (2) would have no effect on enteroviruses. Higgins (3) however feels that the system carries out both primary sedimentation and biological stabilization. Virus reduction would therefore occur by auto or bio-flocculation, in a manner similar to that of an activated sludge process, as observed by Clark and Kabler (4). Limited support for this viewpoint was obtained in South Africa (5) where it was found that at least 60,000 units of polio virus (Mahoney strain) were inactivated in a period of 20 days when added to material from a lagoon.

The apparent lack of information in this important area of waste treatment has prompted the following investigation into the efficacy of pathenogenic virus removal from domestic sewage treated in an oxidation lagoon.

MATERIALS AND METHODS

The following experiments were carried out using poliomyelitis virus, Sabine Type 3 (Connaught Medical Research Laboratories, University of Toronto). Experimentation involved - 1) a model oxidation lagoon, 2) effluent materials from nearby municipal lagoons, 3) untreated domestic sewage and 4) pure culture of Chlorella pyrenoidosa Chick.

Model Lagoon

The material which served as the daily influent for the model lagoon consisted of 55 litres of untreated domestic sewage plus a one litre suspension of the polio virus in distilled water. The resulting mixture, which had a virus concentration of 10^3 units/ml of influent was stirred gently and stored at 5°C. while being pumped into the model lagoon at a constant rate during a 24 hour period.

The lagoon was contained in a painted steel tank approximately 4 feet by 8 feet by 8 feet, and had a volume of 5,525 litres when operated at a depth of 3 feet, with a designed turnover of 14 weeks. The tank was illuminated both by outside light, entering via nearby windows having a northern exposure, and by a bank of 8 Sylvania F48T12-Gro VHO Gro Lux lamps, mounted about 2 feet above the lagoon surface. Surface light intensity at the centre of the tank with 100 per cent cloud cover at mid-day was 200 foot-candles. The lagoon was exposed to an artificial light/dark cycle of 18:6 hours. The light period began at 5 a.m. and ceased at 11 p.m.

The point of influent entry was located 1 foot above the floor of the tank midway between the sides and 6 feet from the tank outlet. Two taps, 1.5 and 3 feet respectively above the floor of the tank, were located on the wall farthest from the point of influent entry.

Effluent from the tank passed out via the upper tap continuously and was collected in a large reservoir during a 24 hour period, which coincided with a daily loading of influent. Effluent samples were obtained from this 24 hour composite solution. After sampling the reservoir was emptied and rinsed thoroughly.

The lagoon operated at a temperature of 20±2°C. in a room having a temperature of 24±3°C.

The following tests were carried out during the operation of the model according to procedures described in Standard Methods (6):

biochemical oxygen demand (BOD)
dissolved oxygen (DO)
total coliforms
fecal streptococci
algae enumeration in Areal Standard Units (ASU)

Fecal coliform determinations were also carried out by filtering aliquots of solutions through a membrane filter having a pore diameter of 0.45 microns (Millipore). The membrane was then transferred to a pad saturated with McConkey Membrane Broth (Oxoid) (7) contained in a petri dish. The plates were incubated in sealed plastic trays at 45°C. in a water bath for 18±2 hours (8), and then counted.

Preparation of the model lagoon was carried out by filling the tank to a depth of three feet with untreated domestic sewage and aerating the material continuously for seven days by means of perforated tubing lying on the bottom of the tank. After five days of aeration two litres of solution from a nearby municipal lagoon, containing algae, were added to the tank. Continuous illumination of the tank with the Gro Lux lamps also began at this time. After another six days 200 litres of water were added to the tank to compensate for evaporation losses. Four days later the tank was again inoculated, this time with 40 litres of solution from the same nearby lagoon.

The first indication of an increasing algal population was evident in two days. After another ten days continuous illumination was replaced by an 18:6 hour light/dark cycle. Two days later, thirty days after the initial filling of the tank with raw sewage experimentation began.

Polio Virus Testing

Isolation of Polio virus was carried out by centrifuging the experimental sample at 3,000 rpm for 20 minutes. Antibiotic was then added to 20 ml of supernatant to a final concentration per millilitre of 500 ug streptomycin, 500 units penicillin, 100 units mycostatin. The sample was thoroughly mixed and allowed to stand for 2 hours at room temperature. Two millilitres of ether were added and the mixture shaken for 15 minutes. The sample was then placed in a separatory funnel and incubated over night at 4°C. Ten millilitres of the acqueous fluid were collected and dialyzed in four changes of distilled water and one in Hawk's balanced salt solution. The dialysate, adjusted to the original volume of 10 ml, was used to inoculate tissue cultures directly or diluted with medium HB597 (modified medium 199).

Rhesus monkey kidney roller tube cell cultures were prepared according to Melnick (9). When the culture was ready for inoculation the maintenance medium was removed by draining and 0.2 ml of sample dialysate of each dilution, from undiluted to 10^{-3} diluted, was added to each of 10 tubes (10 tubes per dilution). For samples expected to have a very low virus titre (eg effluent from the model lagoon), 0.5 ml of undiluted dialysate was used per tube. Adsorption was carried out at 37°C. for one hour before 2 ml of maintenance medium HB597 was added. The inoculated tubes were then incubated at 37°C., and examined daily. Cultures were recorded positive when cytopathenogenic effects became apparent. The tissue culture infections, 50 per cent dose ($TCID_{50}$), were calculated according to Reed and Muench (10), and virus isolates identified by a type specific serum neutralization test (9).

Municipal Lagoon Effluent

Effluent from three municipal lagoons receiving mainly untreated domestic sewage were obtained in mid-December. The ponds had become covered with a thin layer of ice, thus making sampling within the lagoons impossible. The pH and algal population of the respective samples was noted. During the experimental run at the laboratory the samples were maintained at $15 \pm 2^{o}C$. and exposed to a 12:12 hour light/dark cycle. Light intensity was 400 foot-candles.

Untreated Domestic Sewage

Untreated domestic sewage used in the following experiments was pumped directly into the laboratory from a sewer main serving a nearby suburban development. The sewage contained little, if any, industrial waste.

Algae

Experiments involving algae were carried out using a pure culture of Chlorella pyrenoidosa Chick. (culture #395, Indiana Culture Collection). The culture was incubated at $20^{o}C.$, in continuous light having an intensity of 400 foot-candles and was grown in Bristol's solution as described by Starr (11).

RESULTS

Model Lagoon

Operational Data: Influent and Effluent. To determine whether the model system was operating with an efficiency comparable to a field lagoon, the influent and effluent were sampled daily and characterized on the basis of biochemical oxygen demand (BOD), suspended solids content, and total and fecal coliforms present. Counts of fecal streptococci were determined three times each week, beginning the fifth week after the experiment began.

The data presented in Table I are based on mean values for the BOD and suspended solids and median values for the bacterial data. Confidence limits at the 95 percent level have also been included (12). Influent values are calculated from the full 20 week period. Effluent values are derived from data during the period 0-14 weeks and 15-20 weeks. The designed turnover was 14 weeks.

The results indicate that a high degree of reduction was attained by the system. Measurements based on the two groups of fecal organisms indicate that in both cases reduction exceeded 99 percent. On the basis of the above tests the lagoon would appear to have been operating in a satisfactory manner.

Operational Data: Lagoon Contents. Conditions in the lagoon were also monitored. Samples were obtained by drawing off the lagoon solution at the lower tap on the tank wall. The dissolved oxygen content at midday, and pH, were determined daily, and algal counts, three times a week. The results of these tests are illustrated in Figure 1 on the basis of weekly means.

The fluctuations in dissolved oxygen, pH, and algal numbers appear to be somewhat related to each other. The predominant algal genera in the lagoon were the "green algae" - Chlorella, Scenedesmus, and Ankistrodesmus. The "blue-greens" consisted mainly of Oscillatoria.

TABLE I

Influent and Effluent Characteristics of the Model Lagoon

Tests	Influent	Effluent			
	0 - 20 weeks	0 - 14 weeks	per cent reduction	15 - 20 weeks	per cent reduction
BOD ppm	59.0 ± 7.0	4.5 ±0.2	92	4.3 ±1.7	93
Susp. Solids ppm	93.0 ± 9.8	24.6 ± 3.0	73	24.0 ± 6.3	74
Total Colif. per 100 ml	$6.3 \times 10^6 \pm 6.0 \times 10^5$	$9.0 \times 10^3 \pm 2.4 \times 10^3$	99	$2.9 \times 10^4 \pm 1.3 \times 10^4$	99
Fecal Colif. per 100 ml	$4.9 \times 10^5 \pm 1.1 \times 10^5$	$8.7 \times 10^2 \pm 2.3 \times 10^2$	99	$1.7 \times 10^3 \pm 6.5 \times 10^2$	99
Fecal Strept. per 100 ml	$4.3 \times 10^3 \pm 1.1 \times 10^3$	21 ± 4.8	99	4.0 ± 1.3	99

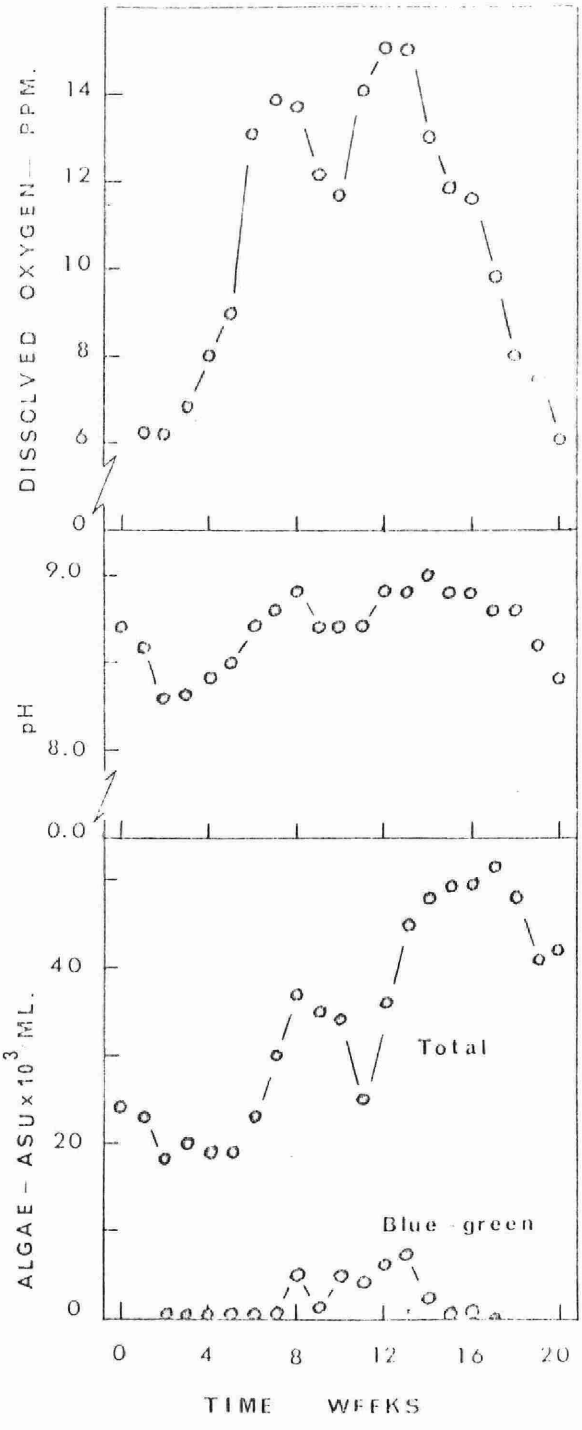

Figure 1 - Characteristics of the Contents of the Model Lagoon - Dissolved Oxygen, pH, Algae.

Virus Experiment: Continuous Loading. In the following experiment the efficacy of the oxidation lagoon to reduce the level of a pathenogenic virus in raw sewage was investigated. A suspension of Sabine type 3 poliomyelitis virus was thoroughly mixed with untreated domestic sewage resulting in an initial virus concentration of 10^3 units/ml of influent per day. Samples tested for the presence of virus included the sewage before addition of virus, lagoon influent six hours after mixing, and lagoon effluent. The sewage, without added virus, was monitored to allow adjustment in the rate of virus loading to the lagoon if necessary. The sequence of sampling and the results are shown in Table ii.

No polio virus was isolated from the untreated sewage during the experiment. A considerable portion of the added polio virus would appear to have been inactivated in the raw sewage before the mixture reached the lagoon. No virus was isolated from the lagoon effluent. Sludge which accumulated on the bottom of the tank was analyzed at the end of 20 weeks and also contained no recoverable virus.

Under the conditions of the experiment the results would suggest that raw sewage is capable of inactivating at least some virus and the remaining virus is removed in the lagoon.

Virus Experiment: Batch Loading. To obtain a more direct estimate of the relationship between the lagoon contents and virus reduction, a 350 ml aliquot of the lagoon solution was evenly divided among seven large test tubes. Six tubes were inoculated with a 1 ml suspension of the Sabine virus with a resulting virus concentration of 10^5 units/ml in the final solution. The tubes were then mounted upright in a wire basket and the basket suspended in the lagoon. After a given period the tubes were removed from the lagoon and tested for recoverable virus.

The results presented in Figure 2 show that

TABLE II

Amount of Added Poliovirus Recovered from Domestic Sewage - Before and After Treatment in the Model Lagoon (Logarithm $TCID_{50}/ml$). Initial Virus Titre- $10^3 TCID_{50}/ml$

Time (Weeks)	Untreated Sewage	Lagoon Influent	Lagoon Effluent	Lagoon Sludge
1	-	1.8	0	-
2	-	2.0	0	-
3	-	1.8	0	-
4	0	1.8	0	-
8	0	1.4	0	-
12	0	1.4	0	-
14	0	1.0	0	-
16	0	1.0	0	-
18	0	1.6	0	-
20	0	1.4	0	0

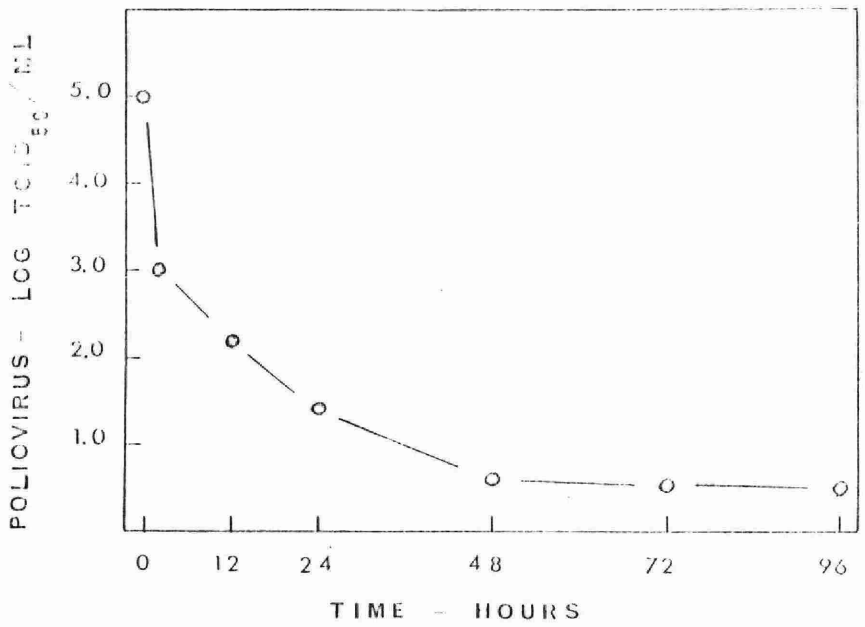

Figure 2 - Effects of the Model Lagoon Contents on Added Poliovirus (Logarithm $TCID_{50}$/ml). Initial Virus Titre - 10^5 TCID/ml.

after a period of 96 hours only about 36 virus units could be recovered from a tube which contained an initial virus concentration of 5 million units. No virus was isolated from the untreated sample. This data provides additional evidence that the model lagoon was capable of reducing the level of virus.

Municipal Lagoon Effluent and Virus

In an attempt to extend the results of the model lagoon to field conditions, samples of three municipal lagoon effluents, obtained in mid-December, were brought to the laboratory and inoculated with poliomyelitis virus. The initial virus concentration of the test samples was 10^5 units/ml. Untreated samples were also tested for their viral content. The results of these experiments, shown in Table III, also include an estimate of the algal population (ASU) and pH.

Considerable variation exists between the virus reduction and lagoon effluents, however in all treatments the amount of reduction in virus titre exceeded that of a virus suspension in distilled water, and in at least one case closely approximated the results obtained in the previous experiment.

Domestic Sewage and Virus

The reduction of virus titre in domestic sewage, noted above, was further investigated by studying the relationships between the supernatant and solids of domestic sewage and virus removal.

A sample of raw sewage, similar to that used with the model lagoon, was separated into two components, supernatant and solids, by centrifugation at 3,000 rpm for 20 minutes. Solids were then resuspended with glass distilled water. Poliovirus was added to aliquots of each portion, the initial virus concentration being 10^5 units/ml. One series was maintained at 4°C. and the other at 20°C. Virus titres were determined immediately after mixing and then at regular intervals. The decrease in titre of a virus suspension in glass distilled water at 20°C. was also noted.

TABLE III

Effects of Municipal Lagoon Effluents on Added Poliovirus (Logarithm $TCID_{50}$/ml) - Initial Virus Titre - 10^5 $TCID_{50}$/ml.

Source	Algae ASU/ml	ph	Time - hours 0	12	24	48	72
Arthur	1523	8.7	3.8	2.4	2.1	1.5	1.0
Bradford	1158	7.9	3.9	3.7	3.3	3.1	2.9
Shelburne	1870	7.8	3.6	3.0	2.5	2.8	2.3
Single Dist. Water	-	-	-	-	-	-	3.1

TABLE IV

Effects of Components of Domestic Sewage on Polio - virus - (Logarithm $TCID_{50}$/ml). Initial Virus Titre- $10^5 TCID_{50}$/ml

Domestic Sewage		Time - Hours 0	6	24	48
Supernatant	4°C.	5.0	5.0	4.6	-
Solids	4°C.	4.8	4.4	4.1	-
Supernatant	20°C.	4.4	4.4	4.0	3.8
Solids	20°C.	4.6	4.2	4.0	4.0
Glass Distilled Water		4.4	4.2	3.6	3.6

The data, presented in Table IV, show that the decrease in virus titre in the sewage series did not exceed the degree of reduction of the suspension in the glass distilled water. These results would suggest that neither component of raw sewage contained materials antagonistic to the virus.

Algae and Virus

As algae comprise a major component of an oxidation lagoon suspension, the relationships between these organisms and virus were investigated in the following experiment. A week old culture of Chlorella pyrenoidosa, cell population about 93,000 ASU/ml, was separated by centrifugation into supernatant and solids in a manner similar to the previous experiment. The cells were then reconstituted with distilled water. Poliovirus was added to aliquots of the two portions, initial virus concentration being 10^5 units/ml. The suspensions were incubated at room temperature during the experiment.

Data shown in Table V indicate that the amount of reduction of virus titre with either component was less than the reduction in the control series in glass distilled water. The algal culture would appear to contain no properties deleterious to the virus.

TABLE V

Effects of Constituents of a Pure Culture of Chlorella pyrenoidosa on Poliovirus (Logarithm $TCID_{50}$/ml). - Initial Virus Titre - $10^5 TCID_{50}$/ml

Algal Culture	Time - Hours					
	0	2	4	6	8	24
Supernatant	4.8	4.8	4.6	5.2	5.0	4.6
Resuspended Algae	5.0	5.0	4.6	4.8	5.0	5.0
Glass Distilled Water	4.6	4.8	4.2	4.4	4.2	4.0

DISCUSSION AND CONCLUSION

The above experimental results have shown that poliovirus present in sewage fed to a model lagoon could not be detected either in the lagoon effluent or in sludge on the bottom of the lagoon (Table II). The lagoon contents have been further shown to be capable of reducing virus levels almost 100,000 fold on a per millilitre basis (Figure 2). Similar experiments with municipal lagoon effluents demonstrated an ability to reduce recoverable virus from 10^5 units/ml to less than 10^3 units/ml in 72 hours (Table III). The constituents of raw sewage and also of a pure algal culture failed to exhibit any antagonism to the virus (Tables IV, V). The relatively innocuous effects of untreated sewage and algae toward enteroviruses has also been noted by Clarke et al (13) and Malherbe (14), respectively.

These later results suggest that the reduction of virus titre, observed with the model lagoon and municipal effluents (Tables II, III, and Figure 2) is not due initially to either a toxic chemical reaction or a biologically oriented pheonomenon. The reduction in virus titre could result from the adsorption of viral particles to flocculating materials, either auto or biological, by a cation-protein reaction suggested by Chan et al (15), and thus be removed by settling. Subsequent degradation of a virus particle might then be expected to result from the action of extracellular enzymes of micro-organisms inhabiting the sludge on the bottom of the lagoon.

While the results of these experiments show that sewage and algae have little direct effect on the virus, it is also apparent that the treatment of raw sewage, which contains polio virus, by passage through the model oxidation lagoon did result in a substantial decrease in the poliovirus titre.

SUMMARY

The efficacy of virus removal by a model oxidation lagoon was investigated by continuous and batch loading experiments. The technique was then extended to experiments with municipal lagoon effluents. The effects of constituents of domestic sewage and of a pure culture of Chlorella pyrenoidosa to reduce polio virus levels were also investigated.

Experimental results show that poliovirus present in sewage fed to the model lagoon could not be detected in either lagoon effluent or lagoon sludge. The lagoon contents were found capable of reducing poliovirus levels almost 100,000 fold. Municipal lagoon effluents reduced poliovirus titre from 10^5 to less than 10^3 units per millilitre. Constituents of raw domestic sewage and of the pure algal culture contained no materials antagonistic to the virus.

Reduction of virus titre in lagoons is probably due to the adsorption of viral particles to flocculants and subsequent settling.

ACKNOWLEDGEMENT

The technical assistance and discussions by personnel of the Connaught Medical Research Laboratories, University of Toronto, particularly Dr. D.R. McLeod and Mr. W. Ing, and also of personnel of the Divisions of Research and Laboratories, Ontario Water Resources Commission, during the study is gratefully acknowledged.

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